

AMENDMENT UNDER 37 C.F.R. 1.116

Serial Number: 09/458,862

Filing Date: December 10, 1999

Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES

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In the Claims

Please amend the claims as follows:

1. (Currently amended) A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, which agent is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, a biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo.
2. (Currently amended) The cryopreservation medium of claim 1, 53 or 54 wherein the cells are peripheral blood lymphocytes or lymphocytes which are activated or genetically modified ex vivo.
3. (Original) The cryopreservation medium of claim 1 that comprises arabinogalactan.
4. (Original) The cryopreservation medium of claim 1 further comprising a cryoprotective agent that penetrates the cell membrane.
5. (Original) The cryopreservation medium of claim 4 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
6. (Currently amended) The cryopreservation medium of claim 1 further comprising a cryoprotective agent other than the arabinogalactan, or a biological or functional equivalent thereof, which does not penetrate the cell membrane.
7. (Original) The cryopreservation medium of claim 1 which does not comprise protein.

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8. (Original) The cryopreservation medium of claim 1 which is infusible.
- 9-10. (Canceled)
11. (Original) The cryopreservation medium of claim 1 wherein the cells are human cells.
12. (Original) The cryopreservation medium of claim 1 wherein the cells are non-human vertebrate cells.
13. (Canceled)
14. (Currently amended) A composition suitable for administration to a human, comprising a suspension of cells in a cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, and a cryoprotective agent that penetrates the cell membrane, wherein the arabinogalactan, or a biological or functional equivalent thereof, is present in an amount of 1% w/v to 40% w/v, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified ex vivo, or a combination thereof, and wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo.
15. (Canceled)
16. (Previously presented) The composition of claim 14 wherein the cells are peripheral blood lymphocytes.

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17. (Previously presented) The composition of claim 14 wherein at least one of the cryoprotective agents is arabinogalactan.
18. (Canceled)
19. (Previously presented) The composition of claim 14 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
20. (Currently amended) The composition of claim 14 further comprising a cryoprotective agent other than arabinogalactan, or a biological or functional equivalent thereof, which does not penetrate the cell membrane.
21. (Previously presented) The composition of claim 14 which does not comprise protein.
22. (Previously presented) The composition of claim 14 which is infusible.
23. (Canceled)
24. (Previously presented) The composition of claim 14 wherein the cells are human cells.
25. (Canceled)
26. (Currently amended) A method for preserving cells comprising:
 - (a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent, that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified

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- ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo; and
- (b) freezing the cell suspension to yield a frozen cell suspension.
27. (Original) The method of claim 26 further comprising thawing the frozen cell suspension under conditions that maintain cell viability.
28. (Original) The method of claim 26 wherein the cells are human cells.
29. (Canceled)
30. (Currently amended) The method of claim 26, 57 or 58 wherein the cells are peripheral blood lymphocytes or lymphocytes which are activated or genetically modified ex vivo.
31. (Currently amended) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and iii) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified ex vivo, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, biological or functional equivalent thereof, results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo.
32. (Original) A frozen hematopoietic cell-containing composition made according to the method of claim 26.

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33. (Original) The cryopreservation medium of claim 5 wherein the cryoprotective agent that penetrates the cell membrane is glycerol.
34. (Original) The cryopreservation medium of claim 33 wherein the concentration of glycerol is about 1% to about 3%.
- 35-36. (Canceled)
37. (Currently amended) A cryopreservation medium comprising a balanced electrolyte solution, at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the balanced electrolyte solution is selected from the group consisting of lactated Ringer's solution, PlasmaLyte-A™, Normosol-R™, Veen-D™, Polysal®, and Hank's balanced salt solution, and wherein the arabinogalactan, biological or functional equivalent thereof, results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo.
38. (Previously presented) The cryopreservation medium of claim 37 wherein the lymphocytes are peripheral blood lymphocytes.
39. (Previously presented) The cryopreservation medium of claim 37 wherein the agent is arabinogalactan.
40. (Previously presented) The cryopreservation medium of claim 37 further comprising a cryoprotective agent that penetrates the cell membrane.

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41. (Previously presented) The cryopreservation medium of claim 40 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
42. (Previously presented) The cryopreservation medium of claim 37 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.
43. (Previously presented) The cryopreservation medium of claim 37 which does not comprise protein.
44. (Previously presented) The cryopreservation medium of claim 37 which is infusible.
- 45-46. (Canceled)
47. (Previously presented) The cryopreservation medium of claim 37 wherein the cells are human cells.
48. (Previously presented) The cryopreservation medium of claim 37 wherein the cells are non-human vertebrate cells.
49. (Previously presented) The method of claim 26 wherein the medium comprises arabinogalactan.
50. (Previously presented) The method of claim 26 further comprising a cryoprotective agent that penetrates the cell membrane.
51. (Previously presented) The method of claim 50 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

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52. (Previously presented) The method of claim 26 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.
53. (Currently amended) A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.
54. (Currently amended) A cryopreservation medium comprising a balanced electrolyte solution incorporating ~~at least one cryoprotective agent that is~~ arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan and glycerol in the medium ~~results~~ result in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.
55. (Currently amended) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, and iii) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly

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isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo.

56. (Currently amended) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) glycerol in amount of 0.5% to about 20%, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified ex vivo, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan and glycerol in the composition ~~results~~ result in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo.
57. (Currently amended) A method for preserving cells comprising: freezing a cell suspension comprising cells and a cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan in an amount of 1% w/v to 40% w/v, and glycerol in amount of 0.5% to about 20%, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan and glycerol in the medium ~~results~~ result in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo.
58. (Currently amended) A method for preserving cells comprising:
- (a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent that is arabinogalactan, in an amount of 1% w/v to 40% w/v, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or

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genetically modified ex vivo, or a combination thereof, and wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified ex vivo; and

(b) freezing the cell suspension at a cooling rate of about 1° to about 10° C/minute to yield a frozen cell suspension.

59. (New) The medium of claim 1, 37, 53 or 54 wherein the post-thaw survival rate is at least about 40%.
60. (New) The method of claim 26, 57 or 58 wherein the post-thaw survival rate is at least about 40%.